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Effect of Imidacloprid and Triadimefon on microbial phosphatase, protease and urease enzyme activities in tomato (*Lycopersicon esculentum* sp.) cultivated soil

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Abstract: The laboratory studies were conducted to resolute the effects of Imidacloprid (insecticide) and Triadimefon (fungicide) singly and in combination on enzymatic activities of soil microorganisms in tomato cultivated soils at different concentrations of 0.2, 0.5 and 0.7 kg per hectare. It was observed that phosphatase, protease and urease activities were elevated noteworthy at 0.5kg per hectare after 10 days of incubation. At lower concentration the activities of these enzymes showed no significant difference from that of control. The combination of the two pesticides led to a pronounced decline of phosphatase and protease enzyme activities at higher concentration. Triadimefon had no effect on urease activity at low concentrations whereas at 0.5 and 0.7 concentrations there was a significant increase when compared to control.

Keywords: Imidacloprid, Soil enzymes, Tomato cultivated soil, Triadimefon

INTRODUCTION

Modern agriculture depends on wide variety of synthetically produced chemicals including insecticides, fungicides, herbicides and other pesticides (Zhang *et al.*, 2011). When a synthetic pesticide is released into the environment, about 0.1% is reaching the target organism, while the remaining 0.99% interferes local metabolism or enzymatic activities, and also affects human health by entering into the food chain which has raised considerable public concern (Ramudu *et al.*, 2011). These pesticides eventually reach the soil and may affect the growth of soil microbial community (Omar and Abdel Sater, 2001). Although these pesticides have been restrictively used, still bioaccumulation is found in soils. Thus, it is required to estimate soil biological responses to the pesticides in terms of soil enzyme activities. When compared to the enzymes from different sources, soil enzymes commonly show particular and peculiar feature (Nasreen *et al*., 2012) in terms of energy transfer, nutrient cycling, environmental quality and crop productivity.

Tomato (*Lycopersicum esculentum*) is a major vegetable crop in Madanapalle, Chittoor district of Rayalaseema region, Andhra Pradesh, India. It is grown in abundance in the district with an average of 35,000 acres producing 3 to 4 lakh million tons per annum and extensively used in fruit processing industries.Pesticides like Imidacloprid and Triadimefon are commonly used for pest control in tomato crop nowadays. Imidacloprid is a systemic nicotinic compound with a potent insecticidal activity against a wide range of pests of vegetable crops (Nasreen *et al.*, 2012) and Triadimefon is a systemic triazole foliar fungicide with a good fungicidal activity (Extension Technology Network, Cornell university). Despite of their potent role in pest control, there is no information available on the interaction effects of imidacloprid and triadimefon in tomato cultivated fields of Madanapalle. Among the various enzymes, phosphatase, protease and urease play important role. Phosphatases mineralize organic phosphorus to inorganic phosphorous. Protease catalyzes the hydrolysis of proteins to poly peptides and oligo peptides to amino acids (Kandeler, 1989). Urease catalyzes the hydrolysis of urea to CO_2 and NH_3. Hence, it is aimed to study the influence of the pesticides singly and in combination on the soil microbial phosphatase, protease and urease enzyme activities in red soil of tomato crop, *L.esculentum.*

MATERIALS AND METHODS

Soil sampling and analysis: Red soil was collected randomly from different sites of tomato cultivated fields of Madanapalle, Chittoor district of Andhra Pradesh, India near the rhizosphere zone (a zone of increased microbial and enzyme activity where soil and root make contact) using a trowel at a depth of 0-12 centimeters and mixed thoroughly to prepare a homogenate composite sample, air dried then passed through 2 mm sieve to remove plant material and other debris. This soil was brought to the laboratory, sealed in bags and stored at 4^0C prior to analysis. The soil was analyzed for various physico-

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chemical parameters viz., sand,silt,clay,P^H,water holding capacity etc. and soil organic matter estimation by dichromate digestion (Walkley and Black, 1934), Micro-kjeldahl method for nitrogen estimation (Jackson, 1971) was done. Unamended and pesticide amended soil samples were slightly alkaline with a pH ranging from 7-8.2 and they were having a medium content of organic matter, soil moisture and nitrogen content. The microbial population was same in untreated and triadimefon treated soil whereas there is slight decrease in the number of microbial population in imidacloprid amended soil.

Pesticides used: The selected pesticides imidacloprid and triadimefon were purchased from Saraswathi agrochemicals, Jammu & Kashmir, and Bayer Crop Sciences, Himatnagar, India.The chemical formula and IUPACs name of these pesticides are as follows:

Imidacloprid: The chemical formula of imidacloprid is $C_9H_{10}ClN_5O_2$ and the IUPAC name is (*E*)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine.It is also known as nicotinamide and a common insecticide.

Triadimefon : The chemical formula of triadimefon (Triazole) is $C_{14}H_{16}ClN_3O_2$ and the IUPAC name of it is 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-one.It is a common fungicide.

Soil incubation: 5, 2 and 1 g. of soil samples were taken in test tubes (25×150 mm) for the assay of phosphatase, protease and urease activities respectively. Stock solutions from selected pesticides were added at the rate of 25, 50, 75 µg per gram soil, which are equivalent to field application rate of 250,500 and 750 gms per hectare respectively. Soil samples without pesticide application served as controls. Soil samples were mixed thoroughly for uniform distribution. Duplicates were maintained at 28 ± 40^0C with 60% water holding capacity (WHC) throughout the experimental period.

Assay of enzymes: The method employed for assay of phosphatase was developed by Tabatabai (1983). Soil samples were transferred to Erlenmeyer flasks and treated with 0.2ml toluene to arrest the enzyme activity. To this 4ml of 0.1 M malate buffer, 2 ml of 0.03 M para nitro phenol (PNP) were added, mixed thoroughly, incubated for 3 hours at 37^0c, and then kept in ice. Then the contents were filtered by passing through Whatmann no. 1 filter paper. To the filtrate, 1ml of 5 mM $CaCl_2$ and 4ml of 0.5M NaOH were added and mixed uniformly. The yellow color developed was read at 405nm in a spectrophotometer.

Ladd and Buttler (1972) method was employed to assay the activity of protease. Soil samples were collected in sterile Erlenmeyer flasks. To these samples, 10 ml of Tris base buffer with 1% sodium caseinate was added .No casein was added to the control. Then 4ml of TCA was added to these samples and centrifuged. To the filtrate, 3ml of 1.4M Na_2CO_3, 1ml Folin Ciocalteu's reagent (FC) were added. The color developed was read at 760nm in a Spectrophotometer.

After 10 days of incubation, duplicate soil samples were withdrawn and urease enzyme activity was assayed using standard method of Fawcett (1960). The soil samples were transferred to Erlenmeyer flasks and 1 ml of 3% urea, 2ml of 0.1 M sodium phosphate buffer were added and kept for 30 minutes of incubation at 37^0C, then cooled at 4^0C for 10 minutes to stop enzyme activity. Then 10ml of 2M KCl was added to the soil samples and centrifuged. 4ml of filtrate was collected. To this filtrate, 5ml of sodium nitroprusside and 3ml of 0.02M hypochlorite solution were added and kept in dark room for 30 minutes. Yellow color developed was assayed at 630nm using a Spectrophotometer.

RESULTS

Phosphatase activity in tomato, *L. esculentum* cultivated soil showed a variable pattern in response to pesticides and their combination after 10 days of incubation. Enzyme activity increased at all the concentrations except at higher concentrations of Triadimefon and Imidacloprid. The combination of these two showed maximum

Table 1. Physico-chemical and microbiological properties of the soil.

Properties	Untreated soil	Soil treated with imidacloprid	Soil treated with triadimefon
Sand %	70	72	72
Silt %	11	12	16
Clay %	5.0	4.8	4.8
pH	8.46	7.89	8.0
Water holding capacity (WHC) ml.g-1 soil %	0.4	0.4	0.4
Organic matter %	0.44	0.38	0.27
Total nitrogen content	0.04	0.19	0.07
Microbial content (in CFU/ml)			
Bacteria	11	9	11
Fungi		7	10

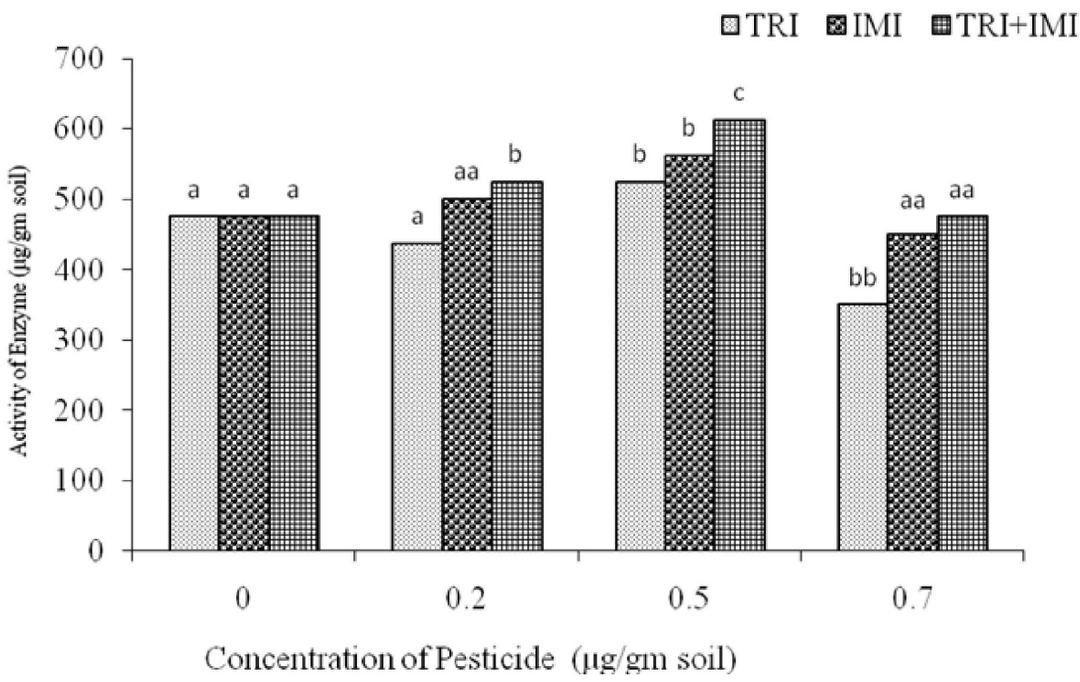

Fig.1. *Showing the phosphatase enzyme activity.TRI-Triadimefon, IMI-Imidacloprid, TRI+IMI-Triadimefon+Imidacloprid. Bars marked by the same letter are not significantly different (P<0.05) from each other according to DMR (Duncan's multiple range) test.*

enhancement of enzyme activity at field application rate (FAR) and there was much difference in the activity compared to control (Fig. 1).

The insecticide, imidacloprid showed much decrease in protease enzyme activity at high concentration. Interestingly this pesticide showed maximum enhancement of enzyme activity at field application rate (FAR) than the others. The combination of the pesticides was stimulatory at all the concentrations. Triadimefon also had a noticeable effect at field application rate (Fig.2). The urease activity was increased at field application rate (FAR) in all the samples treated with triadimefon, imidacloprid and the combination of the two pesticides. There was a significant stimulation of urease activity by the application of triadimefon at field application rate. With imidacloprid and the combination of two pesticides, there was a dramatic decrease both at the lower and higher concentrations in comparison to the control (Fig.3).

DISCUSSION

The tomato, *L.esculentum* cultivated soil samples were slightly alkaline with a pH ranging from 7-8.2 and they were having a medium content of organic matter, soil moisture and nitrogen content. The microbial population was same as that in untreated and triadimefon treated soil whereas there is slight decrease in the number of microbial population in imidacloprid amended soil.Persistence of pesticide residues in the soil may have a significant effect on soil microbial functions such as the activity of enzymes which are directly related to soil health, fertility and also to the removal of contaminants. Among the soil enzymes, phosphates, proteases and urease play an important role. Phosphatases mineralize organic phosphorus to inorganic phosphorous (Omar and Abdel Sater, 2001). Phosphatases play a key role in P mineralization in soil and it is important to know the response of these enzyme activities to the changes in the environmental factors, agricultural management and pollution (Nannipieri *et al.,* 2011). The hydrolysis of proteins, first phase in mineralization of soil organic nitrogen is dependent on enzymes, which are synthesized by plants and soil microorganisms. Protease plays a key role in this step ·Urease plays a key role in hydrolysis of urea into NH_{4+} ions and CO_2.(Tabatabai, 1983) and influences the availability of plant utilizable forms of nitrogen in soils with nitrogen carriers especially urea.

The present study revealed that there was a stimulatory effect in relation to protease activity, which was also supported by Jaya madhuri and Rangaswami (2009) . Protease activity was accelerated up to field application rate (FAR) of imidacloprid, triadimefon and their combination. Kumar and Prakash (1993) demonstrated that two herbicides, Thiobencarb and butachlor reduced protease activity at 25ppm but increased it at 75 ppm. On the other hand, Trifuralin and Pendimethrin progressively reduced the protease activity with increasing concentration (Pahwa and Bajaj, 1999)

The present results of urease showed significant stimulation by fungicide, Triadimefon treatment. Addition of insecticide at field application rate (FAR) significantly

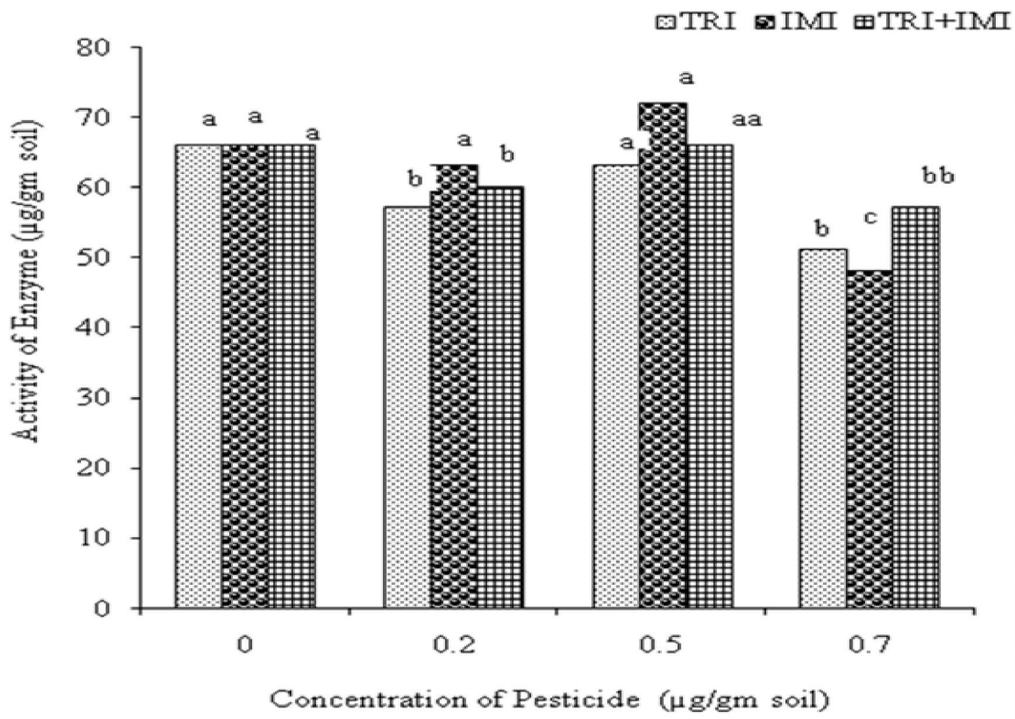

Fig. 2. *Showing protease activity. TRI-Triadimefon, IMI-Imidacloprid, TRI+IMI-Triadimefon+Imidacloprid. Bars marked by the same letter are not significantly different (P<0.05) from each other according to DMR (Duncan's multiple range) test.*

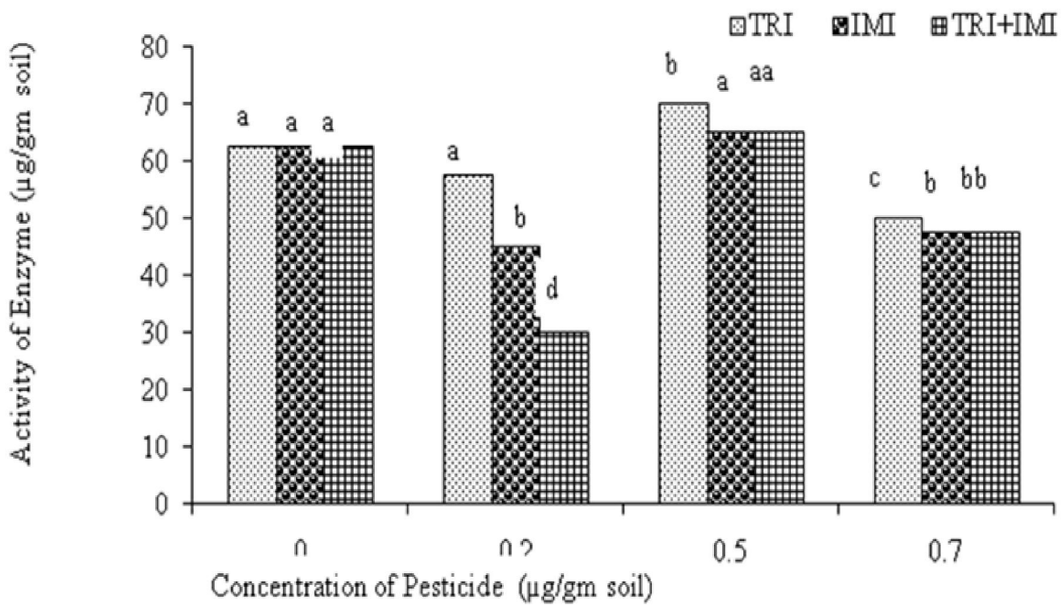

Fig.3. *Showing urease activity. TRI-Triadimefon, IMI-Imidacloprid, TRI+IMI-Triadimefon+Imidacloprid. Bars marked by the same letter are not significantly different (P<0.05) from each other according to DMR (Duncan's multiple range) test.*

increased urease activity at 10 days incubation. The activity was inhibited at higher concentration. Sannino and Gianfreda (2001) observed that urease was relatively unaffected by several pesticides such as Glyphosate and Paraquat. When the treatment effects of diazinon or imidacloprid were compared for the control there were no significant positive or negative effects of insecticides (Ingram *et al.*, 2005).The present studies showed pronounced effect of phosphatase at field application rate by the combination of the two pesticides.

Conclusion

It was concluded that the activities of three enzymes were not affected by the pesticides when applied at the recommended levels (FAR) to the agriculture system. The inhibition of enzymatic activities especially at higher concentration in soils indicated reduced microbial activity. It is attributed to the influence of added chemicals or their metabolites formed in soils during incubation.

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